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12: Hospital Equipment]

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*Indian Standard*  
SPECIFICATION FOR  
THIN WALLED GLASS CAPILLARY PIPETTES

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INDIAN STANDARDS INSTITUTION  
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG  
NEW DELHI 110002

# Indian Standard

## SPECIFICATION FOR

### THIN WALLED GLASS CAPILLARY PIPETTES

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# *Indian Standard*

## SPECIFICATION FOR

## THIN WALLED GLASS CAPILLARY PIPETTES

### 0. FOREWORD

**0.1** This Indian Standard was adopted by the Indian Standards Institution on 16 September 1985, after the draft finalized by the Medical Glass Instruments and Appliances Sectional Committee had been approved by the Consumer Products and Medical Instruments Division Council.

**0.2** Originally thin walled glass capillary pipettes were introduced for microhaematocrit procedures and even today they are used in biological and medical sciences predominantly, but with advancement of science and technology they have gained wide spread acceptance in many other fields of science where micro-technique works are involved.

**0.3** The thin walled glass capillary pipettes are generally used as disposable one. For practical purpose the word 'disposable' expressed in this standard and described in these pipettes means 'one time use only'. A caution note should also be added as 'any individual or institution who reuses these pipettes must bear full responsibility for its safety and effectiveness'.

**0.4** Assistance has been derived from the following international standards:

ISO 384-1978 Laboratory glass wares — Principles of design and construction of volumetric glassware

ISO 1769-1975 Laboratory glass ware — Pipettes — Colour coding

**0.5** For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960\*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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\*Rules for rounding off numerical value ( *revised* ).

## 1. SCOPE

**1.1** This standard specifies general requirements and test for thin walled glass capillary pipettes used generally in haematological and biological work.

**1.2** These pipettes which are heparinized, siliconized or plain are for 'one time use only' for delivering micro quantities of liquid as per methods given in the standard.

## 2. CLASSIFICATION

**2.1** This standard covers the following types of pipettes:

- a) *Type 1* — Where dimensions are necessary adjunct without reference to volumetric capacity.
- b) *Type 2* — Where specific volumetric capacity is necessary adjunct when filled end to end.
- c) *Type 3* — Where specific volumetric capacity is necessary adjunct when filled up to ring mark(s) graduation.

## 3. REFERENCE TEMPERATURE

**3.1** The standard reference temperature, that is, temperature at which the pipette is intended to contain its nominal volume (nominal capacity) is 27°C.

## 4. UNIT OF VOLUME

**4.1** The unit of volume is cubic millimetre (mm<sup>3</sup>) for which the name microlitre may be used.

NOTE — The term microlitre (μl) is commonly used as a special name for cubic millimetre (mm<sup>3</sup>) in accordance with International System of Units (SI).

## 5. MATERIALS

**5.1 Glass** — The pipette shall be manufactured from glass of suitable properties as required for the end use.

**5.2 Heparin** — Heparin used for coating the inner surface of the pipette shall be either sodium, lithium or ammonium salt as required for end use.

**5.2.1** When sodium or lithium salt heparin is used for coating the inner surface of the pipette, the heparin potency shall be 100 USP units per milligram of sodium or lithium salt heparin used.

**5.2.2** When ammonium salt heparin is used for coating the inner surface of the pipette, the heparin potency shall be 60 USP units per milligram of ammonium salt heparin used.

## 6. DESIGN, CONSTRUCTION AND WORKMANSHIP

**6.1** Construction of pipette shall be one straight piece, open at both ends, without lips or constriction. The design dimensions and permissible variations shall be in accordance with respective tables and figures.

**6.2** Any cross-section of the pipette taken in a plane perpendicular to the longitudinal axis shall preferably be circular.

**6.3** The pipette shall be free from defects that noticeably detract from their appearance or impair their serviceability. The pipette shall be free from foreign matter, loose or embedded line or chips that effect the bore or stains when viewed under normal room lighting.

**6.4** The pipette ends shall be cut approximately at 90° to the longitudinal axis and shall not be cracked or have jagged ends or chips that enter the bore of the pipette.

**6.5** Ends of the pipette may be fire polished.

### 6.6 Ring Mark/Marks

- 6.6.1** a) Ring mark for colour coding ( that is, identification of volumetric capacity to contain in the pipette ).
- b) Ring mark/marks up to which the liquid to contain normal volumetric capacity.

**6.6.2** Ring marks for colour band as per **6.6.1(a)** shall be sufficiently deposited on the glass to identify the pipette as to its nominal volumetric capacity in accordance with Fig. 2 and the colour of the band shall be in accordance with the Table 1.

**6.6.3** Other ring marks of **6.6.1(b)** shall be sufficiently deposited on glass to enable the setting of meniscus and thickness shall be in accordance with Fig. 2 and these ring mark(s) shall be only in black colour.

**6.6.4** Heparin coated pipette shall have red or purple colour band applied to the glass at location specified in Fig. 2.

### 6.7 Reading and Setting the Meniscus for Type III

**6.7.1** *Reading Liquid Meniscus* — Reading is made on the lowest point of the meniscus. In order that the lowest point may be observed, it is necessary to place a shade of some dark material immediately below and behind the meniscus, which renders the profile of the meniscus dark and clearly visible against a light background.

**TABLE 1 COLOUR OF BAND OF PIPETTE**  
( Clause 6.6.2 )

NOMINAL CAPACITY	COLOUR CODE
5	White
10	Orange
20	Black
25	2 White
44.7	Green
50	Green
100	Blue
200	Red

**6.7.2 Setting a Liquid Meniscus** — The setting of the meniscus shall be performed by the methods detailed below. Wherever practicable, the meniscus should descend the position of setting:

- a) *Method 1* — The position of the lowest point of the meniscus with reference to the graduation line is horizontally tangent to plane of the upper edge of the graduation line. The position of the meniscus is obtained by having the eye in the same plane of the upper edge of the graduation line.
- b) *Method 2* — The position of the highest point of the meniscus with reference to the graduation line is such that it is in the plane of the middle of the graduation line. The position of the meniscus is obtained by making the setting in the centre of the ellipse formed by the graduation line on the front and the back of the pipette as observed by having the eye slightly above the plane of graduation line. The setting is accurate if the eye is lowered and the ellipse narrows, the highest point of the meniscus remains midway between the front and rear portions of the graduation line. By this method, it is possible to observe the approach of the meniscus from either above or below the line to its proper setting.
- c) The difference between meniscus positions resulting from the above methods of adjustment is the volume equivalent of one half the thickness of the graduation line. The difference between the two methods of adjustment is unlikely to exceed 0.4 percent volumetric error from stated capacity and a correction can be calculated where necessary.

**6.8 Heparin Coating** — Heparin coated pipette inner surface shall be evenly coated with either sodium, lithium or ammonium as required for end use either entirely or up to calibration line.

**6.8.1** When entire inner surface is coated with sodium or lithium heparin minimum of 5 units and maximum of 10 units of heparin activity shall be present.

**6.8.2** When inner surface is coated with sodium heparin up to calibration line a minimum of 2 units and maximum of 5 units of heparin activity shall be present.

**6.8.3** When entire inner surface is coated of Type II or up to calibration line of Type III with ammonium heparin a minimum 2 units of heparin activity shall be present.

**6.8.4** Heparinized pipette may appear cloudy. This phenomena is present with drying of heparin within a capillary and shall not effect the functional use.

**6.8.5** *Fluidity* — Coagulation of the sheep plasma or human blood shall not be evident when viewed under normal room lighting and tested in accordance with **8.3**.

## 7. DIMENSIONS

**7.1** Dimensions of Type I pipette shall be in accordance with Table 2 and Fig. 1.

**TABLE 2 DIMENSIONS OF TYPE I**

LENGTH mm $\pm$ 0.5 mm	OUTSIDE DIAMETER mm	WALL THICKNESS mm
32	0.8 $\pm$ 0.2	0.20 + 0.03 - 0.02
75	1.4 $\pm$ 0.30	0.20 + 0.03 - 0.02
90	1.4 $\pm$ 0.30	0.20 + 0.03 - 0.02
100	1.4 $\pm$ 0.30	0.20 + 0.03 - 0.02

**7.2** Nominal capacities and dimensions of Type II shall be in accordance with Table 3 and Fig. 1.

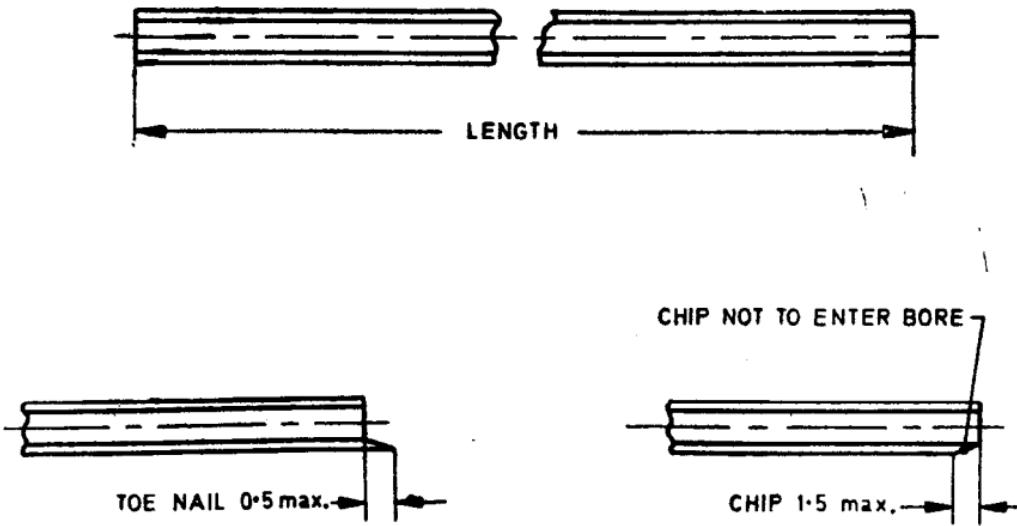


FIG. 1 PIPETTES, TYPES 1 AND 2

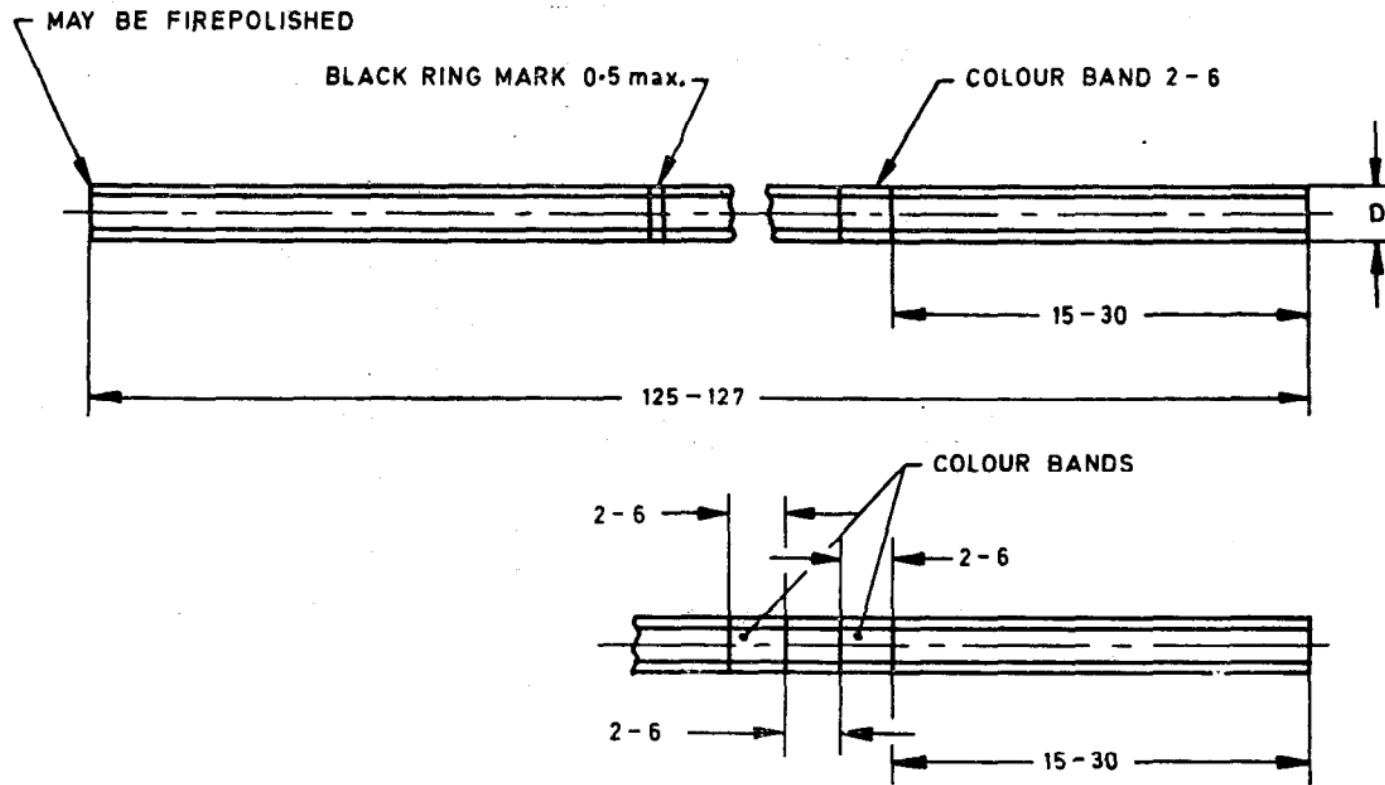
**TABLE 3 NOMINAL CAPACITIES AND DIMENSIONS OF TYPE II**  
( Clause 7.2 )

NOMINAL CAPACITY	MINIMUM LENGTH	MINIMUM OUTSIDE DIAMETER	MINIMUM WALL THICKNESS	MAXIMUM VOLUMETRIC DEVIATION	
				Accuracy	Coefficient Variations
µl	mm	mm	mm	± Percent	± Percent
1	20	0.5	0.2	1.5	2.0
2	20	0.5	0.2	1.2	1.5
3	20	0.6	0.2	1.2	1.5
4	20	0.6	0.2	1.2	1.5
5	20	0.6	0.2	1.2	1.5
10	20	0.6	0.1	1.0	1.2
20	20	0.6	0.1	1.0	1.2
25	30	0.6	0.1	1.0	1.2
30	30	0.7	0.1	1.0	1.2
35	30	0.7	0.1	1.0	1.2
40	30	0.8	0.1	1.0	1.2
44.7	30	0.8	0.1	0.8	1.0
50	30	1.0	0.1	1.0	1.2
60	40	1.0	0.1	1.0	1.2
80	50	1.1	0.1	1.0	1.2
85	50	1.1	0.1	1.0	1.2
100	50	1.3	0.1	1.0	1.2
200	80	1.4	0.1	1.0	1.2
250	80	1.5	0.1	1.0	1.2

**7.3** Nominal capacities and dimensions of Type III shall be in accordance with Table 4 and Fig. 2.

**TABLE 4 NOMINAL CAPACITIES AND DIMENSIONS OF TYPE III**

NOMINAL CAPACITY	GRADUATION MARK(S) AT	MINIMUM OUTSIDE DIAMETER	MINIMUM WALL THICKNESS	MAXIMUM VOLUMETRIC DEVIATION	
				Accuracy	Coefficient Variation Percent
µl	µl	D mm	mm	± Percent	Percent
5	1-2-3-4-5	1.0	0.2	1.0	1.5
5	5	1.0	0.2	1.0	1.5
10	10	1.0	0.2	0.5	1.0
20	20	1.3	0.2	0.5	1.0
25	25	1.3	0.2	0.5	1.0
44.7	44.7	1.3	0.2	0.5	1.0
50	50	1.3	0.2	0.5	1.0
100	100	1.3	0.2	0.5	1.0



All dimensions in millimetres.  
FIG. 2 PIPETTES, TYPE 3

## 8. TESTS

**8.1 Accuracy of Dimensions** — Accuracy of dimensions for all types of pipettes shall be in accordance with the relevant tables.

**8.1.1 Uniformity of Inside Diameter** — The bore of the pipette shall be uniform within the given diameter in relevant table and shall not vary in excess of 0.025 mm in the total length.

**8.1.2 Uniformity of Outside Diameter** — The outside diameter shall be uniform within the given diameters in relevant tables and tapering in total length of the pipette shall not be in excess of 0.02 mm.

### 8.2 Maximum Volumetric Deviation

**8.2.1 Volumetric Accuracy** — The volumetric accuracy and coefficient of variation shall not exceed the limits given in the respective tables.

NOTE — The definition of volumetric accuracy is expressed as the expected distribution of mean volumes around the normal volume. The definition of coefficient of variation is expressed as the expected distribution of individual volumes around the normal volume.

**8.2.2 Capacity Test for Type II** — A dry pipette and a vessel of distilled water shall be allowed to stand at room temperature of 27°C for two hours. The pipette shall be weighed dry and weight indication recorded. The same pipette shall then be filled with distilled water, by capillary action with specific care to remove all water from the exterior of pipette with dry cloth or gauge. The pipette with water content, shall then be reweighed and the weight indication recorded. The recorded weight indication for the dry pipette shall be subtracted from the recorded weight indication for the pipette filled with distilled water. The difference is representing the balance weight indication reading for the contained water.

**8.2.3 Capacity Test for Type III** — A dry pipette and a vessel of distilled water shall be allowed to stand at room temperature of 27°C for two hours. The pipette shall be weighed dry and weight indication recorded. The same pipette shall then be filled with distilled water, by capillary action and adjusted to the ring mark in accordance with 6.7 with specific care to remove all water from the exterior of pipette with dry cloth or gauge. The pipette with water content, shall then be reweighed and the weight indication recorded. The recorded weight indication for the dry pipette shall be subtracted from the recorded weight indication of the pipette filled with distilled water. The difference is representing the balance weight indication for the contained water.

NOTE 1 — The balance weight reading thus determined as per 8.2.2 and 8.2.3 represents the mass, not corrected for air buoyancy, which is unnecessary in these particular cases.

NOTE 2 — To accurately perform the test of 8.2.2 and 8.2.3 the reliability of the weighing instrument should be confirmed against a known standard and weighing instrument discrepancy shall not exceed as follows:

Normal Capacity of Pipette	Discrepancy
1 to 5 $\mu$ l	0.001 mg or better
10 to 250 $\mu$ l	0.01 mg or better

**8.2.4 Calculation of Volume** — The volume 'V' of the pipette from weighing is calculated in accordance with IS : 8897-1978\*.

**8.2.5 Capacity Deviation for Single Pipette** — In accordance with testing methods outlined in 8.2.2 and 8.2.3 the capacity deviation shall be ascertained as follows:

$$\text{Capacity deviations, percent} = \frac{(V - V_1) \times 100}{V_1}$$

where

$V$  = capacity at reference temperature, and

$V_1$  = nominal capacity of pipette.

**8.2.6 Capacity Deviations for a Minimum of 30 Pipettes** — Thirty pipettes taken at random from manufactured lot, shall be tested in accordance with 8.2.2 and 8.2.3. The volumetric deviation from the 30 pipettes shall be ascertained as follows as per accuracy percent and coefficient of variations:

$$\text{Accuracy, percent} = \frac{100 \times (\bar{V} - V_1)}{V_1}$$

where

$\bar{V}$  = mean of sample measurements, and

$V_1$  = nominal capacity of the pipette.

*Coefficient of variations*

$$\text{Coefficient of variation, percent} = \frac{100s}{V}$$

where

$$s = \sqrt{\frac{\sum (V_1 - V)^2}{n - 1}}$$

where

$V_1$  = individual sample measurement,

$V$  = mean of samples measurement, and

$n$  = number of pipettes measured.

*Alternative method*

*Precision:* Precision can be measured by the standard deviation  $s$

$$s = \sqrt{\frac{\sum (x_1 - \bar{x})^2}{n - 1}}$$

where

$x_1$  = individual value obtained,

\*Tables for calibration and method of verification of volumetric glassware.

$\bar{x}$  = mean value  $\left( \bar{x} = \frac{\sum x}{n} \right)$ , and

$n$  = number of values obtained.

The variation coefficient (  $V$  percent ) is the standard deviation of the mean value expressed in percent can be calculated as:

$$V, \text{ percent} = \frac{s.100}{\bar{x}}$$

**Accuracy:** The accuracy may be determined by the deviation of the arithmetical mean value ( actual value ) from the specified value (  $d$  ).

$$d = \bar{x} - \mu_0$$

(  $\mu_0$  = specified value ).

A comparison is obtained by the percentage of deviations abbreviated to  $R$  percent.

$$R, \text{ percent} = \frac{(\bar{x} - \mu_0) - 100}{\mu_0} = \frac{d.100}{\mu_0}$$

It is possible to define both precision and accuracy by mathematical characteristics, provided that distribution is normal, that is, the curve graphically representing the values is a bell shaped curve.

**8.3 Fluidity Test for Heparinized Pipettes** — Heparinized pipettes shall be tested by one of the following methods.

**8.3.1 Sheep Plasma Test** — The test shall be conducted initially by preparing recalcified sheep plasma by the following process:

- Sheep plasma shall be prepared in accordance with Indian Pharmacopoeia ( assay for sodium heparin ),
- 10 millilitres ( 10 ml ) of the prepared sheep plasma shall be added to 2.0 millilitres ( 2 ml ) of the one percent calcium chloride solution used in heparin assay. The sheep plasma and calcium chloride solution shall be mixed well.

**8.3.2 Controls** — Sample of both the plain sheep plasma and calcified sheep plasma shall be used as controls in accordance with the following:

- Positive control* — A plain ( non-heparinized ) pipette shall be filled with recalcified sheep plasma.
- Negative control* — A heparinized pipette shall be filled with plain sheep plasma.

Immediately after the preparation of the recalcified sheep plasma, the pipette shall be filled by immersing the tips in the recalcified sheep plasma and holding the pipette at such an angle as to facilitate quick filling. The pipette shall be filled to within 5 mm ( in the calibrated pipette from datum mark end ) from the other end and placed in a horizontal position. At the end of one hour period, the pipettes containing plasma

shall be inspected for evidence of coagulation by carefully snapping off segments of pipettes in approximately 25 mm lengths, and placed on a flat surface ( use a dark background to facilitate observation and composing with control sample ). Coagulation has occurred if the sheep plasma become opaque or if a fine fibrin thread is noted.

**8.3.3 Human Whole Blood Test** — Human whole blood may be used in lieu of sheep plasma by following the steps outlined in 8.3.2. Blood of known donor that does not have clotting mechanism deficiencies shall be used as a control.

**8.4 Heparin Content Test** — The heparin content in the pipette shall be determined by the method for assaying heparin in Indian Pharmacopoeia or other acceptable methodology that will correlate and provide equivalent test results. The result obtained shall represent the heparin content on the inner surface of the pipettes. No heparin from the outside surface of the pipettes shall enter the test sample.

**8.5 Resisting to Centrifugal Test** — Pipettes which are claimed to be used with centrifugal machine shall be such that no breakage results when pipettes are tested as follows.

**8.5.1** The pipettes shall be filled to capacity with distilled water and sealed and suspended or set in centrifuge. The centrifuge shall be accelerated gradually to a speed of 12 000 rev/min. The centrifuge shall run at this speed for 4 minutes only and then allowed to stop without using the brake.

## 9. REUSE OF PIPETTES

**9.1** Heparinized pipettes are for single use only. Other pipettes may be reused by cleaning them with two possible methods given below:

*Method 1* — After the pipette is used, insert the pipette into the conical aperture of the rubber stopper on the pipetting aid such that the end of the pipette to emerge at the other end. There is ventilation aperture in the rubber cap to facilitate un-impaired air circulation and the pipette is thus automatically re-filled by capillary action. Once the pipette is full from end to end, empty the pipette by closing the air vent with finger and nipping the rubber cap. The process is repeated for flushing out, using a diluent, a reagent or a sterilizing fluid. It may be necessary to rinse out pipette several times when higher-viscosity liquids are used.

Instead of rubber stopper being used it can be done with sufficient long rubber tube and mouth piece ( known as pipetting aid ) for blow out.

**Method 2** — Pipette is held between thumb and forefinger ( or with a pair of tweezers ) and allowed to be filled up by capillary action. It is then emptied by putting in into a stoppered test tube. This stopper tube contains either a diluent, a reagent or a sterilizing fluid. The tube is closed with stopper. The vigorous shaking of the tube will rinse the pipette.

## 10. INSCRIPTIONS AND MARKINGS

**10.1** The following inscription shall be marked on the pipette unit container package:

- a) Dimensions where capacity is not an adjunct or a number indicating the nominal capacity, adjacent or subject to this number, the symbol 'µl' to indicate the unit in terms of which the pipette is graduated.
- b) The inscription '27°C' to indicate the standard reference temperature.
- c) For heparinized pipettes a lot or control number shall be indicated and this number shall be traceable to the origin of raw material purchases of the manufacturer record.
- d) A statement on expected heparin salt and expected units of heparin with an expiry date ( regarding potency of heparin ) claimed by the manufacturer ).
- e) The manufacturer name or trade-mark.
- f) Actual figures for accuracy and coefficient of variation.
- g) Type of glass material used in fabrication of the pipettes.

**10.1.1** The package of pipettes may also be marked with the ISI Certification Mark.

**NOTE** — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution ( Certification Marks ) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions, under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

## 11. PACKING

**11.1** The pipettes may be packed as agreed to between the purchaser and the supplier.

# INTERNATIONAL SYSTEM OF UNITS ( SI UNITS )

## Base Units

QUANTITY	UNIT	SYMBOL
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

## Supplementary Units

QUANTITY	UNIT	SYMBOL
Plane angle	radian	rad
Solid angle	steradian	sr

## Derived Units

QUANTITY	UNIT	SYMBOL	DEFINITION
Force	newton	N	1 N = 1 kg.m/s <sup>2</sup>
Energy	joule	J	1 J = 1 N.m
Power	watt	W	1 W = 1 J/s
Flux	weber	Wb	1 Wb = 1 V.s
Flux density	tesla	T	1 T = 1 Wb/m <sup>2</sup>
Frequency	hertz	Hz	1 Hz = 1 c/s (s <sup>-1</sup> )
Electric conductance	siemens	S	1 S = 1 A/V
Electromotive force	volt	V	1 V = 1 W/A
Pressure, stress	pascal	Pa	1 Pa = 1 N/m <sup>2</sup>